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RESEARCH ARTICLE

INCIDENCE OF *FLT3-ITD* GENE MUTATIONS AMONG PAKISTANI PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS: A PRELIMINARY STUDY

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ABSTRACT: *Background: FLT3* mutations are common genetic changes reported to have prognostic significance in acute leukemia. Fms-like tyrosine kisnase-3 (FLT3) belongs to class-III tyrosine kinase family and plays an important role in proliferation and differentiation of hematopoietic stem cells. The present study investigated the prevalence, distribution pattern in different cytogenetic groups and association with clinical parameters in Acute Lymphoblastic Leukaemia (ALL) patients. Methods: *FLT3/ITD* mutation was studied in Pre-B ALL (n=82) and Pre-T ALL (n=29) patients by PCR in exons 14 and 15 of *FLT3* gene. Results: *FLT3/ITD* was detected in 7.3 % of Pre-B ALL patients. However, no *FLT3/ITD* mutation was detected in Pre-T ALL patients. Prevalence of *FLT3/ITD* (9.5%) among pediatric (<15 years) patients was high with normal cytogenetics (n=18). In patients with t (9:22) translocation (n=22) and hyperdiploidy (n=3), *FLT3/ITD* mutation was detected in 9.5% and 67% patients respectively. No statistical significant relationship was found between *FLT3/ITD* mutation and clinical features like age, WBC, PLT Count and Hb level. Conclusion: This is the first report investigating *FLT3/ITD* mutation prevalence in ALL patients from Pakistan. It is important to screen this mutation in certain cytogenetic subgroups of ALL patients to further assess their role in patient overall survival and targeted treatment therapy.

KEY WORDS: ALL, *FLT3/ITD*. Pakistan, PCR

INTRODUCTION:

FLT3 receptor (FMS-like tyrosine kinase-3 receptor) is a member of extracellular receptors on hematopoietic precursors and belongs to the class III tyrosine kinase receptor family. *FLT3* affect the proliferation and differentiation of hematopoietic progenitor cells and is an independent negative prognostic factor[1]. Two major mutations in this gene are most common, 1) insertion of tandem duplication into exon 11 and exon 12 in the wild-type *FLT-3* produces internal tandem duplication (ITD), and 2) a mis-sense point mutation or small insertions or deletions within the activation loop of the second tyrosine kinase domain (TKD), called the TKD mutation[2] .

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FLT3 mutations are one of the most commonly reported somatic alterations in AML but little work has been done on these mutations in ALL. *FLT3* is rarely mutated in leukemic lymphoblasts in adult and pediatric ALL.[3,4,5] However, *FLT3* mutations are relatively common among the cytogenetic subgroups of hyperdiploidy and mixed-lineage leukemia (MLL) translocation[6]. An overall low frequency (1-8%) has been reported in childhood ALL. However, a higher incidence has been reported among those with MLL gene rearrangement and high hyperdiploidy.[7,8] In adult ALL, *FLT3* mutations are even rarer[9]. In Pakistan, very little data is available about *FLT3/ITD* prevalence, clinical features and outcomes of ALL patients and this study is the first one describing incidence of *FLT3* mutations in large number of ALL patients. Aim of this study is to identify the clinical features (WBC Count, Hg and PLT count associated with this mutation. This study also aims to identify distribution pattern of this mutation in different cytogenetic groups in our ALL patients. The incidence of *FLT3* mutations in pediatric leukemia is of particular interest due to the several promising *FLT3* inhibitors currently under development[10].

MATERIALS AND METHODS:

Five ml blood samples of 111 (82 of Pre-B ALL and 29 of Pre-T ALL) diagnosed ALL patients were collected from Institute of Nuclear Medicine and Nuclear Medicine Lahore, Pakistan. Informed consent was taken from ALL patients. DNA was isolated from 200 µl whole blood by using genomic DNA extraction kit (Favorgen, Taiwan) according to the manufacturer's protocol. The PCR amplification of *FLT3* gene was done by using the gene specific primers and cycling conditions described elsewhere[11].The results were interpreted based on the appearance of additional bands as compared to wild type using known molecular weight marker. Data was assessed by using Statistical Package for Social Sciences (SPSS) version 16. Chi-Square and Fisher Exact test used for the analysis of data at significant level 0.05.

RESULTS:

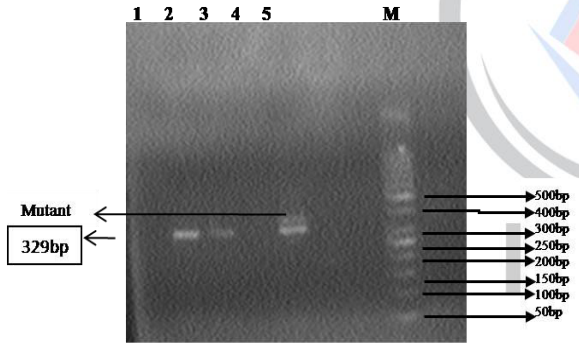
Characteristics	B-Cell ALL	T-Cell
	(n=82)	(n=29)
Age (Years)		
< 15	32	11
16-30	36	10
> 30	14	8
Median(Range)	17.5 (3-53)	18 (4-58)
Hb		
<10mg/dl	61	17
>10mg/dl	21	12
Median(Range)	8.50 (3.6-16.8)	9.2 (6.5-15.7)
WBC $\times10^3$/µl		
<50×10^3	61	24
>50×10^3	21	5
Median(Range)	8.50 (3.6-16.8)	9.2 (6.5-15.7)
Platelet Count $\times10^3$/µl		
<150×10^3	71	18
>150×10^3	11	11
Median(Range)	38×10^3/µl (1-617)	66×10^3/µl (6-482)
Gender		
Male Paediatric	25	9
Adult	38	12
Female Paediatric	7	2
Adult	12	6
Cytogenetics		
Normal	18	10
Translocation t(4:11)	2	0
Translocation t(9:22)	2	0
Tri/del/others	22	6
Hyperdiploidy	3	1
Not available	16	12

Table1: Clinical characteristics of ALL Patients

Among 111 blood samples collected from ALL patients, 82 were of Pre-B ALL and 29 were of Pre-T ALL. Pre-B ALL patients (N=82) were characterized by frequent expression of CD10 (42%) and CD19 (90%) antigen. In pre-B ALL patients, 63 were male and 19 were females. In Pre-T ALL, 21 were males and 8 were females. The median age of Pre-B ALL patients was 17.5

years (range 3-53 years). In females, the median age was 19 years (range 6-45 years) while in males it was 17(range 3-53 years). Majority of the patients were adult (61.53 %) while 38.46 % patients were children. The median WBC of Pre-B ALL was $8x10^3/\mu l$ (range $0.1\text{-}256.3x10^3$). The other clinical characteristics at presentation and cytogenetic analysis of the patients in the studied group are summarized (Table-1).

Cytogenetic analysis in 82 Pre-B ALL patients was performed in only 66 patients. Among these, 18 (27.27%) patients were cytogenetically normal with 46 number of chromosomes. Among other cytogenetic abnormalities, translocations t(4;11) was found in (n=2) 3% patients, t(9;22) in (n=21) 32% patients, Hyperdiploidy/tetraploidy in (n=3) 5% patients and 33% were positive for deletion, trisomies etc (n=22) (Table-1). Majority of the Pre-T ALL 10/29 (35%) patients were cytogenetically normal. Other frequent cytogenetic abnormalities found were trisomies/deletions /others (Table-1).



M: DNA ladder (50bp)
Lane 1: Control without template
Lane 2 and 3: Wild *FLT3* gene 329bp
Lane 4: Negative control
Lane 5: Patient sample with *FLT3* gene mutation

Figure.1

Screening for ITD mutations in exons 14 and 15 in *FLT3* gene was performed in 82 patients. *FLT3/ITD* mutation was detected in 7.3 % of Pre-B ALL patients (Figure1). The prevalence of *FLT3/ITD* in pre ALL pediatric (1-15 years) patients was 9.4 % and 6% in adults (>15 years) respectively. In males (n=63), prevalence of *FLT3/ITD* mutation was 8% and in females (n=19) it was 5.3%. In Pre-T ALL patients, no *FLT3/ITD* mutation was detected. The presence of *FLT3/ITD* mutation was also studied in different cytogenetic groups in 66 pre B ALL patients. Prevalence of *FLT3/ITD* mutation was 11% in patients having normal cytogenetics (n=18). In other cytogenetic groups, namely t(9;22) (n=21) and hyperdiploidy (n= 3), *FLT3/ITD* mutation was detected in 9.52% and 67% patients respectively.

DISCUSSION:

FLT3 gene mutations, particularly *ITD* in AML is the most frequent somatic alterations in AML. Their presence is associated with poor prognosis in AML. *FLT3* mutations are also found in adult and pediatric ALL, but their incidence is much rarer than in AML [8,9,12].The main aim of this study was to establish the prevalence of *FLT3-ITD* mutations among childhood leukemia patients in Pakistani population. In this study, prevalence of *FLT3/ITD* mutations was 7% among 82 Pre-B ALL patients. In another reported study, one (4%) out of 25 ALL patients were positive for *FLT3/ITD* [13]. In another report, involving larger number of samples of diagnosed leukemia patients, the prevalence of *FLT3/ITD* was 7% [14]. The prevalence of *FLT3* in other reports was as high as 9% and 8% in Japan and Sweden respectively[15]. Lower prevalence was reported in Greece, UK and Japan with 1-3.5 %[15]. So, in our study similar prevalence rate was found showing *FLT3* is not very common among our patients suffering from ALL.

In our study, prevalence of *FLT3/ITD* mutation was (9.4%) in 32 pediatric patients which is found higher as compared to other studies reporting lower incidence in patients <15 years of age.

Prevalence of *FLT3/ITD* was reported as 3.3% in sixty pediatric patients[14] whereas in another study of 517 pediatric leukemia cases, the prevalence of *FLT3* mutation was 12.3% for AML and 2% for ALL having age<15 years[14]. Similarly prevalence for *FLT3/ITD* was reported in other studies on pediatric ALL patients[7,15]. This may be due to difference in biology of disease which needs to be investigated further. Similarly, frequency of *FLT3/ITD* mutation among 50 adults patients (>15 years) in our study was observed as 6% . A few studies have reported *FLT3* mutations among adult ALL patients at a very low frequency [12, 17]. In our study, no statistical significance was found between different clinical features like WBC count and *FLT3* mutation status (Table1). No significant statistical relationship between WBC count and *FLT3* mutation has also been reported in other studies [3]. However, one study revealed that the *FLT3/ITD* is associated with high WBC count [13].

In ALL patients, MLL and hyperdiploidy has been identified as subtype of ALL that often harbors *FLT3* mutations[18]. Three hyperdiploid cases, in our study, (6 , 8, 10 years old.) was detected with *FLT3/ITD* mutation. Other studies also reported the presence of *FLT3/ITD* mutation in hyperdiploid cases but does not show any effect on the prognosis [16]. Among other cytogenetic groups, 2/21 Philadelphia positive patients were found positive for *FLT3/ITD* mutation whereas other studies reported no incidence of *FLT3* mutation in this cytogenetic entity in ALL patients[15]. Although patients with hyperdiploidy often harbors *FLT3* mutations as reported in literature and current study has similar results. It is unclear at this stage whether patients with hyperdiploidy ALL might be considered as candidates for therapy with *FLT3* inhibitors. This will require larger studies of MLL and hyperdiploid ALL samples, but it is important to note that all three patients with hyperdiploid ALL in this study harbored FLT3 mutation. The presence of *FLT3* mutations in these cases suggests that *FLT3* inhibition may represent a therapeutic opportunity in at least a subset of patients with ALL.

Among 29 Pre-T ALL, no *FLT3/ITD* mutation was detected in cytogenetically normal patients. Some larger studies have also reported a low frequency of *FLT3/ITD* and/or *FLT3/D835* mutations ranging from 3.3% to 5.5% among T-ALL patients.[19]

In conclusion, current data demonstrated the low prevalence of *FLT3-ITD* mutations in our population. An important question is whether or not the presence of *FLT3* mutations in ALL has prognostic significance. A definitive answer to this question could be investigated in further studies involving larger patients of different cytogenetic subgroups as candidates for therapy with newly described small-molecule FLT3 inhibitors.

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